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Pedometer readings for estrous detection and as predictor for time of ovulation in dairy cattle

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Abstract

The objective of this study was to study the relationship between increase in number of steps measured by pedometers, behavioral estrous parameters and time of ovulation, in order to investigate whether the number of steps can be used as a tool for estrous detection and as a predictor for time of ovulation. In total, 63 ovulations were observed in 43 Holstein-Friesian cows. Different behavioral signs of estrus were observed at 3 h intervals. Cows were equipped with pedometers, which stored number of steps in 2 h time periods and pedometer estrus alerts were defined using different algorithms and thresholds. The percentage of behavioral estruses also detected by pedometers measurements, ranged between 51 and 87% for all estrous periods. When only estrous periods where taken into account in which more than one animal was in estrus, detection percentages increased up to 95%. Number of steps taken during the estrous period was higher when more animals were in behavioral estrus at the same time, and number of steps taken during the estrous period was also higher for primiparous cows compared to multiparous cows. Ovulation occurred 29.3 ± 3.9 h after onset of increased number of steps (ranging between 39 and 22 h) and 19.4 ± 4.4 h after the end of increased number of steps (ranging between 35 and 12 h). The intervals were not influenced by the number of animals that were in estrus at the same time or by parity. In conclusion, pedometers can detect estrus accurately and appear to be a promising tool for prediction of ovulation and hence could be a tool for improving fertilization rates.

Keywords: Cattle; Ovulation time; Pedometer; Activity; Estrus

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1. Introduction

Methods to detect estrus include, e.g. visual observation, changes in body temperature, changes in vaginal mucus resistance, recording of mounting activity and also increase in number of steps around estrus [1]. Activity (measured by pedometers) of dairy cows during estrus was first studied in the early 1950s of the last century [2]. That study showed that the estrous period in dairy cows is characterized by an increased number of steps. Later research showed that the increase in number of steps is a promising tool for accurate detection of estrus [1,3], a prerequisite for good insemination results. Most studies utilizing pedometers, however, have focused on improving efficiency of estrous detection [4] and not on improving fertilization rates.

The chance of fertilization highly depends on the interval from insemination to ovulation. When cows are inseminated too early, chances are that sperm is aged by the time ovulation occurs and cannot fertilize the ovum anymore [5]. And when insemination takes place too late, chances are that because of the ageing of the egg, fertilization and formation of a viable embryo is not possible anymore [6]. Therefore, insemination time should be based on time of ovulation rather than on detection of estrus.

If time of ovulation can be predicted by an increase in number of steps, pedometer readings could be a tool for improving fertilization rates. The present study investigated the relationship between increase in number of steps measured by pedometers during behavioral estrus and time of ovulation to see if increase in number of steps can be used as a tool for estrous detection and as predictor of time of ovulation.

2. Materials and methods

The experiment was conducted at the experimental dairy farm ‘‘de Ossekampen’’ at Wageningen University and Research Centre, The Netherlands. The Ethical Committee for Experimentation with Animals (Wageningen, The Netherlands) approved the experimental protocol.

2.1. Animals, feed and housing

Data were collected from a herd of approximately 70 lactating Holstein-Friesian cows. Forty-nine of these animals showed estrus and a subsequent ovulation at least once during the experimental period. Parity of these 49 animals varied between one and six and the animals were 100.0 ± 65.8 (mean ± S.D.) days in milk, with a range of 17–370 days. Animals were fed an ad lib mixture of grass silage, corn silage and mineral supplements, and concentrates according to production level (Central Animal Feed Bureau (CVB)-norms, 2000). The animals were housed in a free stall with slatted floor and cubicles. The animals were milked by an automated milking system (Liberty, Prolion, Vijfhuizen, The Netherlands). Estimated 305-day milk production was 8297 ± 1334 kg (mean ± S.D.).

2.2. Measurement of number of steps

For activity measurements, animals were equipped with pedometers (Nedap Agri B.V., Groenlo, The Netherlands) on a front leg. The pedometer recorded the number of steps a cow made in 2 h time periods (0–2 a.m., 2–4 a.m., . . ., 10–12 p.m.). Receivers were placed by the entrance and exit of the automatic milking system and the data were transferred to a computer. Approximately 18 days of pedometer measurements around behavioral estrus were analyzed for animals that showed visual signs of estrus (Section 2.4); onset and end of increased number of steps around visual estrus were evaluated using different methods and thresholds (Section 2.3).

2.3. Calculation of an increase in number of steps

Increase in number of steps (measured by a pedometer) was calculated using two different methods, one based on the median number of steps and the other based on the standard deviation of the average number of steps. In both methods, the number of steps taken in a 2 h time period was compared with the number of steps taken in the same time period during the 10 preceding days. Comparisons were done per 2 h time period, because of the presence of a diurnal pattern in number of steps during diestrus (Fig. 1).

In the median method, the number of steps taken in a particular 2 h time period was divided by the median number of steps of the 10 preceding days. Using this approach, a ratio was calculated for every 2 h time period. If this ratio exceeded a threshold, either 10.0 for one period (MED10) or 5.0 for two consecutive periods (MED5), this was defined as an actual increase in number of steps and designated as an estrus alert based on pedometer readings (pedometer estrus alert).

In the second method based on the standard deviation, the mean and standard deviation of the number of steps was calculated for the 10 preceding days. If the number of steps for a particular 2 h time period exceeded a threshold, i.e. the mean plus 2 (S.D. 2), 2.5 (S.D. 2.5), 3 (S.D. 3) or 3.5 (S.D. 3.5) times the standard deviation of the preceding 10 days, this was

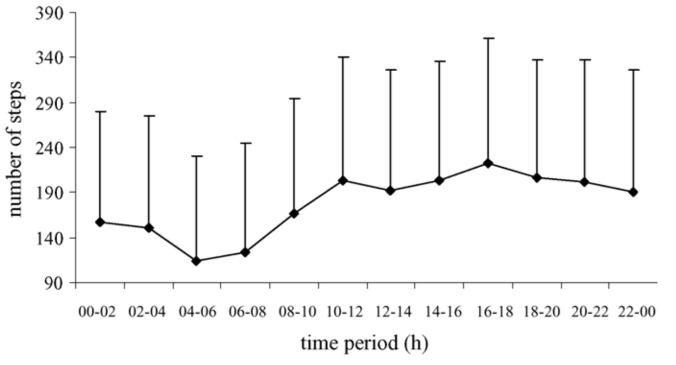

Fig. 1. Number of steps taken in 2 h time periods (mean) for all animals and days, periods of estrus excluded ($n = 1419$–1455). The model also included the class-variable cow ($p < 0.05$) and the interaction between period and cow ($p < 0.05$). The bars show the 95% confidence limits.

defined as an actual increase in number of steps. If this increase occurred for two consecutive periods, this was defined as a pedometer estrus alert. A pedometer estrus alert was defined as correct, when it was accompanied by behavioral estrus (Section 2.4) and ovulation was confirmed (Section 2.5). A pedometer estrus alert was defined as false, when it was not accompanied by a behavioral estrus. A pedometer estrus alert was defined as missed, when behavioral estrus (followed by ovulation) was not accompanied by a pedometer estrus alert. In other words, during one estrous cycle, there was either a correct or a missed pedometer estrus alert, and there could be zero, one or several false pedometer estrus alerts. Fig. 2 shows the activity pattern of an individual animal; day 0 is the day of behavioral estrus followed by ovulation. The dotted line indicates the calculated threshold

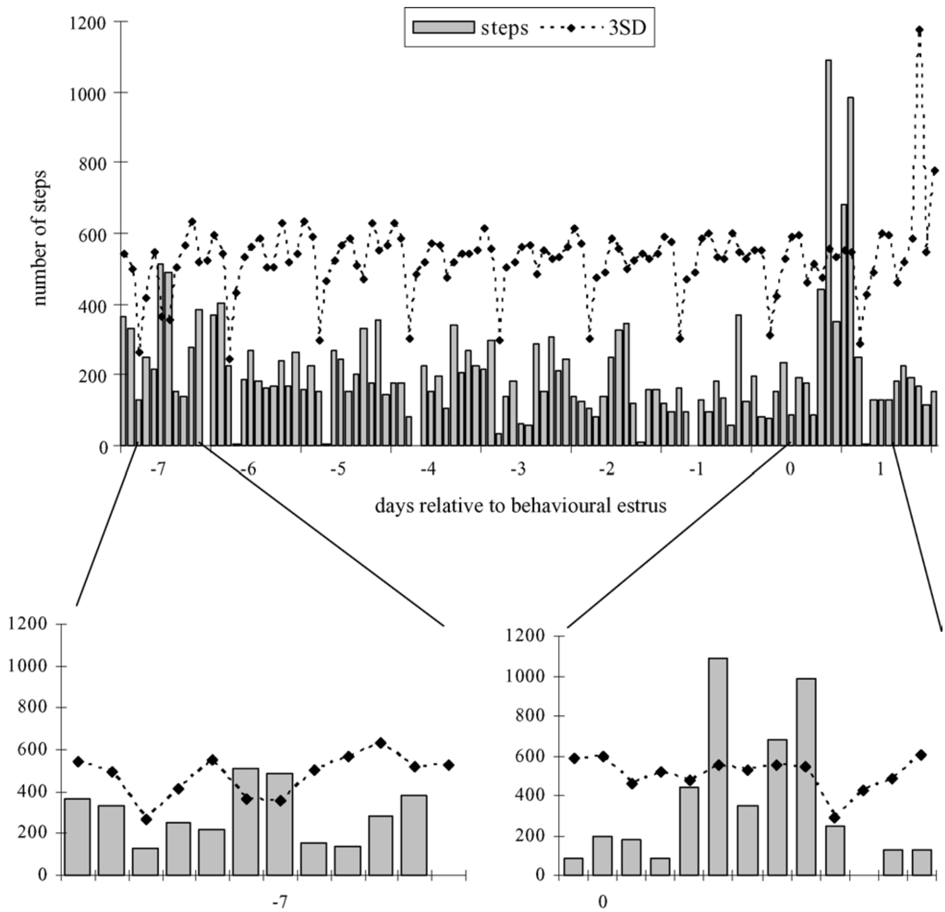

Fig. 2. The activity pattern of an individual animal. The grey bars are the actual number of steps taking in 2 h periods. The dotted line shows the threshold for S.D. 3, which is the mean of the preceding 10 days for that 2 h period plus three times the standard deviation of that mean. When the grey bars exceed the dotted line two consecutive times, this is considered a pedometer estrus alert. This animal had a false pedometer estrus alert on day −7 (enhanced) and a correct pedometer estrus alert on day 0 (enhanced, day 0 = day of behavioral estrus).

for increase in number of steps (using S.D. 3). This animal had a false pedometer estrus alert on day −7 (the continuous line exceeds the dotted line two consecutive times) and a correct pedometer estrus alert on day 0. Onset of estrus based on pedometer readings (pedometer estrus) was defined as the first time at which an actual increase in number of steps was found (beginning of the 2 h time period). End of pedometer estrus was defined when at least two consecutive time periods no increase was found. It was defined as the last time at which an actual increase in number of steps was found (end of the 2 h time period).

2.4. Visual observation of estrous behavior

Two observers monitored behavior simultaneously every 3 h for 30 min (at 8.00, 11.00, 14.00, 17.00, 20.00, 23.00, 2.00 and 5.00 h) from an elevated chair, which did not disturb the animals. Each observer observed half of the stable and after an observation period the results of the observers were summed. Estrus was defined according to Van Eerdenburg et al. [7]. Each time an animal displayed a behavioral estrous sign, the assigned number of points was recorded (Table 1). If the sum of points during consecutive observation periods exceeded 100, the animal was considered to be in behavioral estrus. Onset of behavioral estrus was defined as the first observation period the animal showed estrous behavior minus 1.5 h. End of behavioral estrus was defined as the last observation period the animal showed estrous behavior plus 1.5 h. The number of animals in behavioral estrus during an observation period was recorded. Data were collected in 23 cases from animals with an estrus, induced by i.m. injection of 15 mg of prostaglandin F2-α analogue (Prosolvin, Intervet, Boxmeer, The Netherlands) when a corpus luteum was present. In 40 cases, data were collected from naturally cycling cows.

2.5. Ultrasound detection of ovulation

The ovaries of the cows were examined rectally using an ultrasound scanner (Scanner 200, Pie Medical, Maastricht, The Netherlands). The scanner was equipped with a

Table 1
Scoring scale for observed estrous signs

Estrous signs	Points
Flehmen	3
Restlessness[a]	5
Sniffing the vulva of another cow	10
Mounted but not standing	10
Resting with chin on the back of another cow	15
Mounting other cows (attempt)	35
Mounting head side of other cows (attempt)	45
Standing heat	100

Each time a behavioral estrous sign is observed, the assigned number of points is recorded (modified after Van Eerdenburg et al. [7]).

[a] Can be recorded only once during an observation period.

7.5 MHz sector transducer. The transducer was inserted into the rectum to examine the ovaries. The reproductive tract was not manipulated or palpated before or during ultrasound examination. Ultrasound examinations started between 8 and 11 h after the end of an observed behavioral estrus. During the first ultrasound examination, each ovary was scanned to determine on which ovary the preovulatory follicle was located. Thereafter, scanning the ovary containing this follicle was continued every 3 h until the disappearance of the follicle, which marked ovulation time [8]. Each time, the diameter of the follicle was measured. Time of ovulation was defined as the first ultrasound the preovulatory follicle had disappeared minus 1.5 h. Six days after estrus, ultrasound examination was performed to determine if a corpus luteum had appeared which confirmed ovulation.

2.6. Statistics

Because ovulation was assessed only in cows that showed behavioral estrus, relationships between pedometer readings and ovulation can only be calculated for cows that showed behavioral estrus and not for cows with silent estrus. Preliminary analyses revealed no differences between natural and induced estruses for any of the parameters; therefore, data of induced ($n = 23$) and natural ($n = 40$) estruses were combined. Influences of 2 h time periods on number of steps outside estrous periods were calculated by means of a multivariate analysis of variance (adjusted for multiple comparisons by Bonferroni), using the Statistical Analysis System (The SAS system for windows V8, 1999). In the model were included time period and cow as class-variables and the interaction between these class-variables. Data on intensity of behavioral and pedometer estrus and intervals between pedometer estrus and time of ovulation were also analyzed by means of a multivariate analysis of variance (adjusted for multiple comparisons by Bonferroni). The factors parity and animals in estrus were used as class-variables, in which parity consisted of two classes (primiparous ($n = 20$) and multiparous ($n = 43$) cows) and animals in estrus consisted of three classes (1 = no other animal in behavioral estrus ($n = 15$), 2 = (partly) simultaneously with at most one other animal in behavioral estrus ($n = 26$) and 3 = (partly) simultaneously with at least two other animals in behavioral estrus ($n = 19$)). Chi-square analysis was used to investigate differences between percentages of correct pedometer estrus alerts for the different classes of animal in estrus. All means are presented as mean $\pm$ S.D., unless otherwise stated. p-Values <0.05 are defined as a significant difference and p-values between 0.05 and 0.1 are defined as a tendency for difference.

3. Results

In total, 63 ovulations were observed in 49 animals. For each ovulation, pedometer readings from 18 to 0 days before behavioral estrus and 1–17 days after behavioral estrus (on average 18.3 ± 2.9 days) were analyzed. Basal number of steps per 2 h time period was on average 177 ± 134, but differed between time periods. The lowest number of steps was found in the early morning (4–8 a.m., see Fig. 1).

3.1. Efficiency of different methods of defining pedometer estrus

Table 2 shows number and percentage of correct, false and missed pedometer estrus alerts for the different methods calculating increase in number of steps. The threshold of S.D. 2 and S.D. 2.5 resulted in the highest percentage of correct pedometer estrus alerts (87%). The methods that use the median (MED10 and MED5) resulted in poor percentages of correct pedometer estrus alerts (51 and 52%, respectively). MED10 also resulted in a high number of false pedometer estrus alerts (80 in 63 estrous cycles). The thresholds of S.D. 2.5, S.D. 3 and S.D. 3.5 resulted in high percentages of correct pedometer estrus alerts (87, 83 and 79%, respectively) and relatively low percentages of false pedometer estrus alerts (17, 8 and 5%, respectively).

Of the 63 behavioral estruses with ovulation, 11 were not detected by pedometer readings using S.D. 3 (missed pedometer estrus alerts). The mean and standard deviation of number of steps taken in the 2 h time periods during 10 days prior to behavioral estrus did not differ between the 52 correct pedometer estrus alerts (mean: 179 ± 50 steps; S.D.: 121 ± 31 steps) and the 11 missed pedometer estrus alerts (mean: 184 ± 40 steps; S.D.: 131 ± 29 steps; $p > 0.1$). In other words, the reason why estrus was not detected by pedometers readings was not because those animals had a different activity pattern 10 days before behavioral estrus. Nine of the 11 missed pedometer estrus alerts did have one 2 h time period with increased number of steps. In other words, there was an increase in activity, but of too short a duration to be considered as pedometer estrus alert.

Using S.D. 3, five times a pedometer estrus alert was given when no behavioral estrus with ovulation occurred (false pedometer estrus alert). Of these five false pedometer estrus alerts, two can be explained by the fact that, the animals were forced to go through a footbath, which increased the number of steps in that particular 2 h period. The remaining three false pedometer estrus alerts cannot be explained by clear events. Mean and standard deviation of number of steps taken in the 2 h period during 10 days prior, did not differ between correct (mean: 179 ± 50 steps; S.D.: 121 ± 31 steps) and false (mean: 168 ± 33 steps; S.D.: 127 ± 41 steps; $p > 0.1$) pedometer estrus alerts. However, the actual number of steps in the 2 h period in which the activity was increased, was significantly lower for false pedometer estrus alerts (477 ± 322 steps) compared to correct pedometer estrus alerts (841 ± 259 steps; $p < 0.05$).

Table 2
Percentage and number of correct and missed pedometer estrus alerts and average number of false pedometer estrus alerts for each threshold of increase of activity ($n = 63$)

Pedometer estrus alert	Correct		Missed		False		
	Number	%	Number	%	Estrus[a] (%)	Average[b]	Range[b]
MED10	32	51	31	49	40	3.2	1–17
MED5	33	52	30	48	14	2.1	1–6
S.D. 2	55	87	8	13	37	1.3	1–3
S.D. 2.5	55	87	8	13	16	1.3	1–2
S.D. 3	52	83	11	17	6	1.1	1–2
S.D. 3.5	50	79	13	21	5	1.0	1

[a] Percentage of all behavioral estruses in which at least one false pedometer estrus alert was present.
[b] Number of false pedometer estrus alerts in estrous cycles with at least one false pedometer estrus alert.

3.2. Relationship between behavioral estrus and increase in number of steps

Table 3 shows characteristics of behavioral and pedometer estrus (using S.D. 3) for primiparous and multiparous animals and for different numbers of animals in behavioral estrus at the same time. Duration of behavioral estrus was on average 2 h longer (11.8 h) compared to pedometer estrus (10.0 h; see Table 3). Primiparous cows had a longer duration of pedometer estrus and were more active during pedometer estrus compared to multiparous cows; the same was true for behavioral estrus. The number of animals in behavioral estrus at the same time did not influence any of the parameters of pedometer estrus, but animals displayed more behavioral estrous signs when more than two cows were in estrus at the same time. Although the number of animals in behavioral estrus at the same time did not significantly influence the number of steps during pedometer estrus, more animals were detected in estrus by the pedometers when more than two animals were in behavioral estrus at the same time (95%) compared to when two animals (85%) or only one animal (67%) was in behavioral estrus at the same time. Total, maximum and average number of steps were positively correlated with total, maximum and average number of points acquired during behavioral estrus (r: 0.32–0.56; $p < 0.05$). Total, maximum and average number of steps were also positively correlated with most of the individual behavioral signs (sniffing, chin resting, mounting and standing heat) displayed during behavioral estrus (r: 0.28–0.46; $p < 0.05$). Only total and maximum number of steps were not correlated with chin resting and average number of steps was not correlated with sniffing. Duration of pedometer estrus was positively correlated with duration of behavioral estrus ($r = 0.53$) and with the number of times sniffing ($r = 0.30$) and standing heat ($r = 0.38$; $p < 0.05$) were displayed.

When behavioral estrus was more intense, the chance that this estrus was detected with the pedometer was higher; animals with a pedometer estrus had a longer duration of behavioral estrus (12.3 $\pm$ 4.1 h) compared to the duration of behavioral estrus for animals with a missed pedometer estrus alert (9.0 $\pm$ 4.7 h; $p < 0.05$). Also, animals that had a pedometer estrus acquired more total (1366 $\pm$ 787 points) and maximum (639 $\pm$ 334 points) number of points for behavioral estrus compared to the total (565 $\pm$ 444 points; $p < 0.05$) and maximum (337 $\pm$ 271 points; $p < 0.05$) number of points for animals that had a missed pedometer estrus alert.

3.3. Relationship between increase in steps and time of ovulation

Fig. 3 shows the distribution of the interval between onset and end of pedometer estrus and time of ovulation. The average interval between onset of pedometer estrus and ovulation was 29.3 $\pm$ 3.9 h (range: 39–22 h), and ovulation took place 19.4 $\pm$ 4.4 h (range: 35–12 h) after the end of pedometer estrus. Interval between onset of pedometer estrus and time of ovulation did not differ between primiparous and multiparous cows and was not influenced by the number of cows in behavioral estrus at the same time. The interval between end of pedometer estrus and time of ovulation was shorter for primiparous cows (16.9 $\pm$ 3.0 h) compared to multiparous cows (20.6 $\pm$ 4.5 h), which was probably due to the longer duration of estrus for primiparous cows (Table 3). The interval between end of

Table 3
Characteristics of behavioral estrus (BE) and pedometer estrus (S.D. 3) for one, two or more than two animals in behavioral estrus at the same time and primiparous and multiparous animals (mean ± S.D. (range))

	Animals in estrus			Parity		Average
Pedometer estrus (S.D. 3)	1 ($n = 10$)	2 ($n = 22$)	More than 2 ($n = 18$)	Primiparous ($n = 16$)	Multiparous ($n = 34$)	
Duration (h)	10.0 ± 4.4	9.0 ± 3.8	11.3 ± 4.5	12.4 ± 4.1a	8.9 ± 3.7b	10.0 ± 4.2
	(6–18)	(4–18)	(4–18)	(6–18)	(4–18)	(4–18)
Total steps[a]	3313 ± 1577	3904 ± 2116	5415 ± 2747	6090 ± 2331a	3455 ± 1792b	4298 ± 2317
	(832–5520)	(1320–8416)	(820–9168)	(2640–9168)	(820–8416)	(820–9168)
Maximum steps[b]	910 ± 329	1114 ± 351	1235 ± 436	1397 ± 383a	980 ± 308b	1113 ± 384
	(312–1344)	(544–1760)	(480–2080)	(784–2080)	(312–1648)	(312–2080)
Average steps[c]	662 ± 218	852 ± 210	929 ± 290	984 ± 234a	773 ± 236b	840 ± 254
	(277–932)	(539–1213)	(410–1524)	(660–1524)	(277–1308)	(277–1524)
Behavioral estrus (BE)	$n = 15$	$n = 26$	$n = 19$	$n = 20$	$n = 40$	
Duration (h)	11.0 ± 4.0	11.1 ± 5.0	13.3 ± 3.4	13.5 ± 5.2y	10.9 ± 3.6z	11.8 ± 4.4
	(6–21)	(3–24)	(9–18)	(3–24)	(3–21)	(3–24)
Total points[a]	810 ± 543a	1097 ± 659a	1751 ± 890b	1621 ± 718y	1038 ± 771z	1232 ± 797
	(114–2035)	(235–3303)	(441–3857)	(185–3150)	(114–3857)	(114–3857)
Maximum points[b]	379 ± 188a	551 ± 283ab	806 ± 394b	791 ± 389a	488 ± 268b	589 ± 342
	(100–690)	(170–1243)	(203–1635)	(100–1635)	(101–1268)	(100–1635)
Average points[c]	217 ± 131y	324 ±197y	405 ± 211z	414 ± 236y	277 ± 160z	323 ± 198
	(57–458)	(117–910)	(139–788)	(62–910)	(57–771)	(57–910)

Different letters (a, b) within a row mean a significant difference for the variable ‘animal in estrus’ or for the variable ‘parity’ ($p < 0.05$). Different letters (y, z) within a row mean a tendency for a difference for the variable ‘animal in estrus’ or for the variable ‘parity’ ($p < 0.1$).

[a] Number of points/steps acquired during the entire estrus.

[b] The maximum number of points/steps acquired during a period during estrus.

[c] The average number of points/steps acquired during a period during estrus.

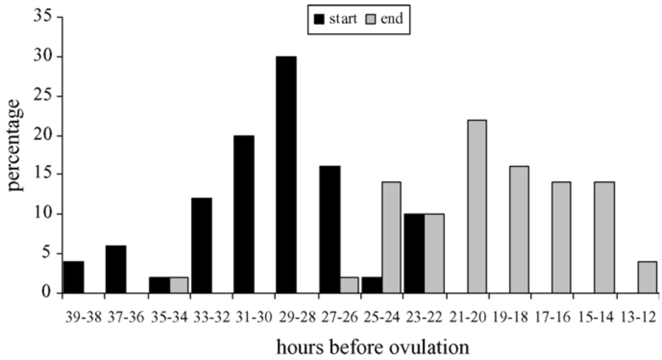

Fig. 3. Distribution of interval between start (n = 51) and end (n = 49) of pedometer estrus and time of ovulation.

pedometer estrus and time of ovulation was not influenced by the number of animals in behavioral estrus at the same time.

4. Discussion

The results of this study show that the increase in the number of steps preceding ovulation can be used to detect estrus and to predict time of ovulation fairly accurate. Ovulation occurred approximately 29 h (ranging from 22 to 39 h) after onset of pedometer estrus (using S.D. 3). If time of ovulation can be predicted, insemination can be timed to get good fertilization results. Not many studies have looked at the effects of different intervals of insemination in relation to ovulation time on conception rates in natural cycling dairy cattle. More then 50 years ago, Trimberger et al. [9] found highest conception rates when cows were inseminated between 7 and 24 h before ovulation. The best conception rate (85.7%) was found when insemination took place between 13 and 18 h before ovulation. If these results still hold, it would mean that insemination should take place between 11 and 16 h after onset of pedometer estrus in our study to get the best fertilization results. Maatje et al. [10] designed an experiment to estimate the optimal time interval from the onset of estrus, determined by pedometers, to artificial insemination. In that study the chance of conception was highest (84.2%) when cows were inseminated between 6 and 17 h after increased pedometer activity; the estimated optimum was at 11.8 h. Unfortunately, time of ovulation was not assessed in that experiment, but when ovulation time would be calculated by the means of the results of our experiment, the results of Maatje et al. [10] would be in agreement with the results of Trimberger et al. [9].

For pedometer readings to be useful as estrous detection as well as predictor of ovulation time, it is important to have a high accuracy and a high detection percentage. The number of steps during 10 days prior to a pedometer estrus gives no indication if a pedometer estrus alert is false or correct. However, looking at the actual number of steps during the pedometer estrus, a low number of steps might indicate that the pedometer estrus alert is false. The number of correct, missed and false pedometer estrus alerts depends on the definition of the threshold for 'increased number of steps'. Different thresholds have been used in experiments to study the increase in number of steps around estrus [10–14]. In our experiment, using a threshold based on the mean of the 2 h time periods during 10 days prior plus several times the standard deviation seemed to give the best results in terms of accuracy and detection. Because the number of steps was not normally distributed, another threshold was calculated using a ratio based on the median of the 2 h time periods during 10 days prior, to see if results would improve. This was not the case; the number of missed and false pedometer estrus alerts was much higher using the method with the median compared to the other method.

Using S.D. 2, S.D. 2.5, S.D. 3 and S.D. 3.5, the percentages of correct pedometer estrus alerts ranged between 79 and 87%, which is a similar range as found in other experiments [11,13,15]. Using S.D. as a threshold, the average number of false pedometer estrus alerts ranged from 1.0 to 1.3 in 5–37% of the 63 behavioral estrus periods. The literature differs considerably in the percentage of false pedometer estrus alerts measured, ranging from 0% [16] to 205% [13] or more [17]. These large differences may be explained by different threshold definitions, different periods (e.g. 12 h instead of 2 h periods) or different management (e.g. different floor surface, no automatic milking system) used in the different experiments.

In our experiment, the method using S.D. 3 (so, comparison of the number of steps in a 2 h period with the mean plus three times the standard deviation of the same 2 h periods for the 10 days prior) showed a good result with 83% correct pedometer estrus alerts and only five false pedometer estrus alerts in only four of the 63 estrous cycles. Two of the five false pedometer estrus alerts can be explained by management factors; at the time the false pedometer estrus alert was given, the cows were forced through a footbath, which increased the number of steps. Koelsch et al. [18] also found that altered routines were a likely source of false estrous indications by pedometer readings. Thus, occurrence of pedometer estrus alerts after alteration of the daily routine should be interpreted with caution. In 9 of the 11 missed pedometer estrus alerts, one 2 h time period with increased number of steps was detected. In other words, there was an increase in activity, but of too short a duration to be considered a pedometer estrus (which requires at least two consecutive 2 h time periods with increased activity). If one period with increased activity would be considered a pedometer estrus, the percentage of correct pedometer estrus alerts would increase to 97%. However, the number of false pedometer estrus alerts would increase tremendously; 79% of the estrous cycles would have one to eight false pedometer estrus alerts with an average of 3.4 per cycle. This shows that the definition used to define increased number of steps (and as a consequence, number of correct, false and missed pedometer estrus alerts) is very important and dependant on several factors, e.g. herd, management, etc.

Because of the presence of a diurnal rhythm in the number of steps [18–20] it should be more precise to store the number of steps in small time periods, and use this in the definition for increased number of steps, as done in our experiment. Past experiments used pedometers that stored the number of steps in time periods of 12 h [11,17,21]. Several experiment have shown that smaller time periods (up to 2 h) improved efficiency (number of correct pedometer estrus alerts) but decreased accuracy (higher number of false pedometer estrus alerts [13,20]). The conclusion of those studies was that number of steps measured for time periods of 12 h was potentially satisfactory for recording cow activities to detect estrus. However, our data show that for 15 of the pedometer estruses, an increase in activity (using S.D. 3 as a threshold) was present for only 6 h or less. These short pedometer estruses would probably have been missed when data storage took place in 12 h time periods. Furthermore, if the increase in number of steps is used to predict time of ovulation, timing of insemination would be based on onset of pedometer estrus. Therefore, it is important to have data storage for small time periods, so timing of insemination in relation to ovulation can be accurate.

Visual observations for behavioral estrous signs were carried out frequent in this experiment (for 30 min every 3 h), which ensured detection of short behavioral estrus periods. In practice some behavioral estruses will be missed when visual observations are carried out less frequent [22]. Also behavioral estruses that occur during the evening and night could be missed by visual observations in practice. Standing heat is the most discriminative sign of estrus [7] but not all cows express this behavioral sign [7,23–25]. When, in practice, estruses are detected only using this behavioral sign, many estruses will be missed. When the three mentioned causes for possibly missed behavioral estruses (short estrus, estrus during night and no standing heat) are taken into account, 28 behavioral estruses (44%) would have been missed in our experiment, 68% of these 28 estruses were detected by pedometers (using S.D. 3) which shows the importance of pedometers for estrus detection.

Estruses can also be 'silent', i.e. no expression of estrous behavior at all preceding ovulation. Because correct and missed pedometer estrus alerts are based on expression of behavioral estrus in this experiment, it is not known whether animals with silent estrus show an increase in the number of steps and would have been detected in estrus using pedometer readings. However, in previous experiments with the same visual observation method, all estruses were detected [22,23].

Several studies show that estrus is more intense when more animals are in estrus at the same time: animals received more points [22], displayed more mounts and stands [22,26,27] and took more steps [28]. In our study, intensity of behavioral estrus was increased when more animals were in estrus at the same time and the number of steps increased when more animals were in estrus at the same time, although the latter increase was not significant ($p = 0.14$ for average number of steps during estrus). When a behavioral estrous sign is displayed intensively (i.e. often during estrus) or the increase in number of steps is more obvious (higher), the chance of detection may also be higher. In our study, overall detection percentage of pedometer estrus was 83%, but when only one animal was in behavioral estrus, significantly less pedometer estruses were detected compared to when two or more animals were in behavioral estrus at the same time. These results indicate that more animals being in estrus at the same time, not only stimulates behavioral signs of estrus, but also increases walking activity during estrus, because of which estrous detection percentages by pedometers increased up to 95%.

Another factor that influenced intensity of estrus in the present study was parity. Behavioral and pedometer estrous durations for primiparous cows were approximately 3 h longer compared to multiparous cows. This is in contrast with studies that found either no difference in behavioral estrous duration between primiparous and multiparous animals [23] or that found a shorter behavioral estrous duration in primiparous cows [22,29]. Also estrus was more intense (more number of points received and number of steps taken during estrus) in primiparous cows compared to multiparous cows. A study that used the same system for behavioral observations found the opposite; multiparous cows acquired on average more points during estrus compared to primiparous cows [22]. Why these differences exist is unclear. To our knowledge, no information is available on the influence of parity on activity patterns.

In conclusion, pedometer measurements can detect estrus accurately and appear to be a promising tool for prediction of ovulation and hence could be a tool for improving fertilization rates. In practice, a daily routine is important for pedometer readings to have a high detection percentage and a high accuracy. In the end it is up to the farmer to choose a threshold that best fits his wishes. If he wants to know for sure that a pedometer estrus alert is correct, there will be more missed pedometer estrus alerts. Vice versa, if a farmer wants to detect as many estruses as possible, the consequence is that there will be more false pedometer estrus alerts. The percentages of false, correct and missed pedometer estrus alerts for the different thresholds in this study only apply to one experimental farm. Further research on factors that can influence activity patterns (e.g. number of animals in estrus, parity, etc.) and validation of the results for different farms (to investigate whether the same thresholds apply to different herds) could be worthwhile to optimize the use of pedometers as a tool for estrous detection and prediction of ovulation.

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